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Neuroanatomy of the grey seal brain: bringing pinnipeds into the neurobiological study of vocal learning

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Abstract

Comparative studies of vocal learning and vocal non-learning animals can increase our understanding of the neurobiology and evolution of vocal learning and human speech. Mammalian vocal learning is understudied: most research has either focused on vocal learning in songbirds or its absence in non-human primates. Here we focus on a highly promising model species for the neurobiology of vocal learning: grey seals. We provide a neuroanatomical atlas (based on dissected brain slices and magnetic resonance images), a labelled MRI template, a 3D model with volumetric measurements of brain regions, and histological cortical stainings. Four main features of the grey seal brain stand out. (1) It is relatively big and highly convoluted. (2) It hosts a relatively large temporal lobe and cerebellum, structures which could support developed timing abilities and acoustic processing. (3) The cortex is similar to humans in thickness and shows the expected six-layered mammalian structure. (4) Expression of FoxP2 - a gene involved in vocal learning and spoken language - is present in deeper layers of the cortex. Our results could facilitate future studies targeting the neural and genetic underpinnings of mammalian vocal learning, thus bridging the research gap from songbirds to humans and non-human primates.

Introduction

Strong evidence for vocal learning in grey seals (*Halichoerus grypus* [1]) makes them an interesting target for comparative neurobiological research. Data from these pinnipeds could provide insights into neural and genetic networks involved in this complex ability in mammals [2–4]. However, cross-species investigation into the neurobiology of vocal learning of distantly related animals is complicated by the presence of large inherent brain differences, for example in morphology and size, unrelated to their vocal learning abilities [5]. To overcome this issue, we performed histological analyses, created a reference neuroanatomical atlas, a magnetic resonance imaging (MRI) brain template, and a volumetric 3D model of the weaned female grey seal brain. This outcome effectively facilitates comparative research by identifying and describing the similarities and differences between the brains of grey seals and other vocal learning and non-learning animals both within and outside the clade Pinnipedia, and the larger overarching suborder Caniformia.

Methods

Two brains were retrieved during post-mortem examination from weaned (one month - one year old) female grey seals. Both animals died of natural causes during rehabilitation (Supplement, *Table S1*). The brains were fixed in formalin and scanned in a 3T MRI scanner. T1 and T2 weighted images were acquired, pre-processed, and segmented to develop 3D models of the grey seal brains (Figure 1) with volumetric measurements of brain regions (*Figure 1*; Supplement, *Table S4-S5*). For one of the two brains, a labelled brain template and neuroanatomical atlas was generated based on the MRI images, dissected slices, and photographs (Supplement, *Figures S2-S8*). Three cortical sections were taken from the same brain for histological examinations (Supplement, extended methodology and *Figure S9-S10*).

Results and discussion

The measured average brain volume of the weaned female grey seals was 204 cm^3. Previously, it was approximated that the adult male grey seal brain was 330 cm^3 and the adult female brain 262 cm^3 [6,7][1]. This makes grey seal brains rather big compared to those of terrestrial carnivores (e.g., polar bears (*Ursus maritimus*, 215 cm^3 [8]) and dogs (*Canis lupus familiaris*, 72 cm^3 [9])), and relatively similar to those of other species within Pinnipedia, such as the California sea lion (*Zalophus californianus*, 307 cm^3 [10]) and the harbor seal (*Phoca vitulina*, females 255 cm^3 and males 271 cm^3 [6]). The volumetric ratio of grey-to-white matter in the weaned female grey seal brain was 2.7. Grey matter volume and white matter volume are related by a logarithmic relationship across all species based on brain size, but this ratio changes across development [11,12]. The values reported here fall within the expected range for the seal's developmental stage.

[1] If volumetric information was not available, but weight measurements were, we converted the weight measurements to volume measurements based on the conversion rules found in [27].

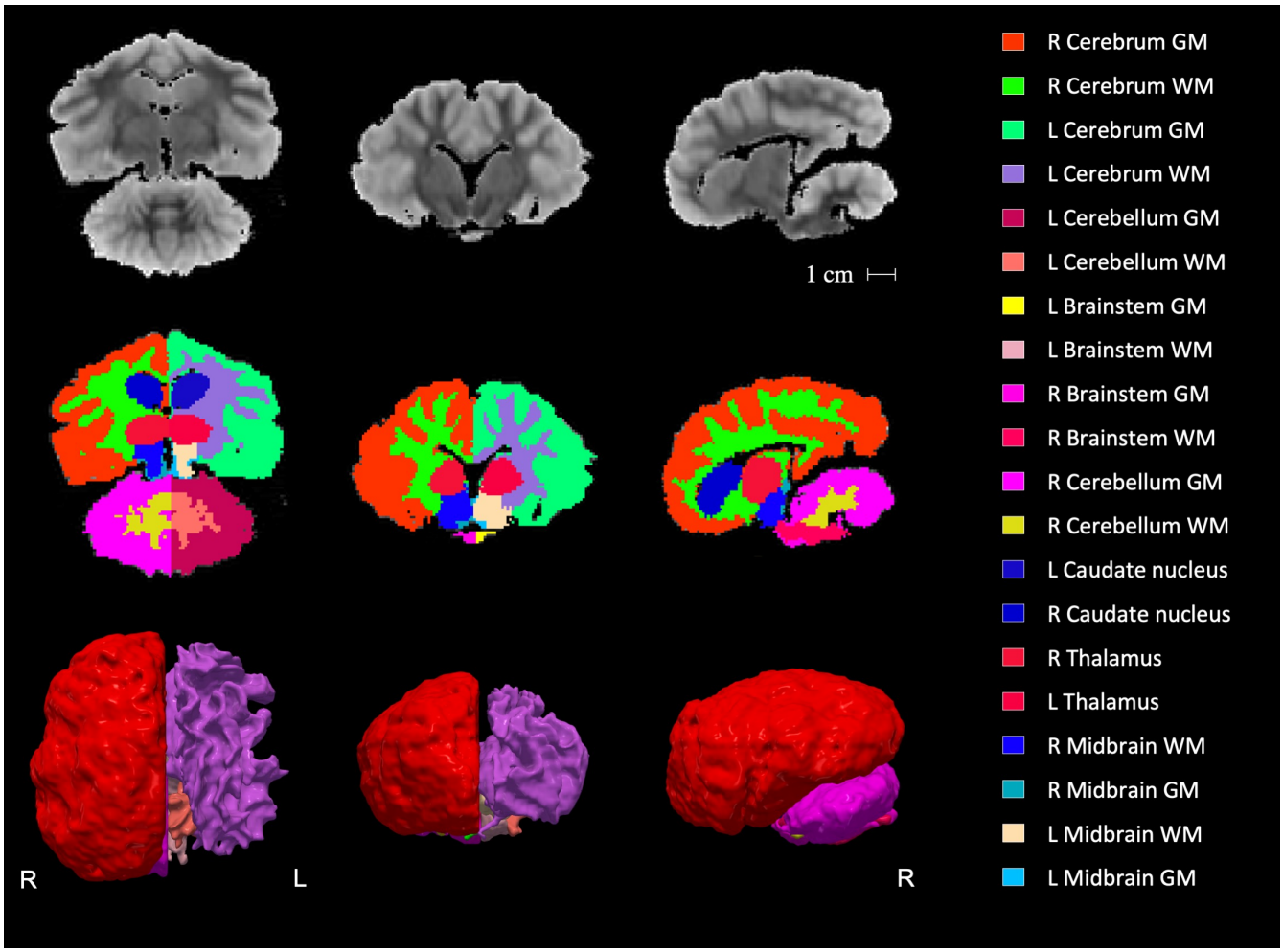

Figure 1. Overview of the grey seal brain template created from Grey Seal Brain 2. From left to right, the transverse (rostral portion on top), coronal, and sagittal (rostral portion on the left) views are shown in radiological convention (right hemisphere on the left side). Top row: T2 FLAIR brain template. Middle row: segmented and labeled brain template. Bottom row: 3D reconstruction of the brain surface from the dorsal (left), frontal (middle), and lateral (right) view showing the grey matter outer boundary on the right hemisphere and the white matter surface on the left. The unlabeled template can be found here and the labeled brain template can be found here. Legends and labels can be downloaded here. Abbreviations: L = Left, R = Right, WM = White Matter; GM = Grey Matter.

The cortex of the weaned female grey seal brains was highly convoluted, with a large number of gyri and sulci (*Figure 2*). Moreover, a great deal of secondary gyration was present (sub-sulci and sub-gyri) in the grey seal brain (Supplement, *Figure S5*). A similar level of gyration has been observed in other pinnipeds, such as the harbor seal and the California sea lion, while lower levels of gyration are present in many terrestrial carnivores with smaller brain sizes, such as canids [10,13]. This complies with the fact that bigger brains are generally more convoluted [14]. The gyration patterns of the grey seal brain largely overlap with those described for harbor seal brains [15–18]. However, they both diverge significantly from the gyration patterns described in terrestrial carnivores, such as canids [13]. The most notable difference is the placement of the sulci delineating the temporal lobe (*Figure 2*).

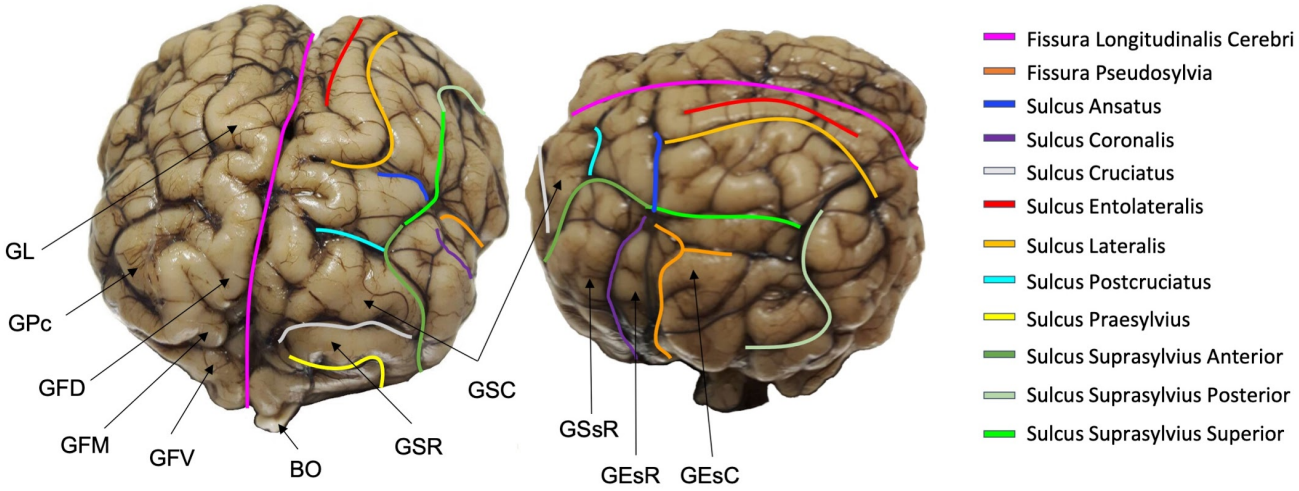

Figure 2. Gross anatomy of the grey seal brain. Left: dorso-frontal surface. Right: lateral surface. Each sulcus is marked in a different color. Abbreviations: BO = Bulbus olfactorius; GEsC = Gyrus Ectosylvius Caudalis; GEsR = Gyrus Ectosylvius Rostralis; GFD = Gyrus Frontalis Dorsalis; GFM = Gyrus Frontalis Medius; GFV = Gyrus Frontalis Ventralis; GL = Gyrus Lateralis; GPc = Gyrus Postcruciatus; GSC = Gyrus Sigmoideus Caudalis; GSR = Gyrus Sigmoideus Rostralis; GSsR = Gyrus Suprasylvius Rostralis.

The weaned female grey seal brains were fairly spherical (i.e., the width and length of the brain were almost identical). This is due to relatively small olfactory bulbs and an expanded temporal lobe. Other pinnipeds share this same brain shape, but other animals within Caniformia do not (e.g., [10,13]). The presence of a well-developed temporal lobe is relatively rare in mammals outside of primates but has previously been observed in a variety of cetaceans and pinnipeds [19]. The temporal lobe hosts the auditory cortex, which is involved in the processing of auditory stimuli, and in humans is involved in speech processing and production [19]. Future studies could further explore the function of the expanded temporal lobe, the auditory cortex and associated circuitry in pinnipeds.

The weaned female grey seal brains had a relatively large cerebellum compared to their total brain size. The average cerebellum size in our current study was 31 cm^3, which corresponded to 15% of the total brain volume (Supplement, *Table S4-S5*). The cerebellum is a key brain structure for motor control and plays an important role in vocal learning [20] and cognition [21]. In humans [22], and in terrestrial carnivores such as canines [9], the cerebellum accounts for approximately 10% of the total brain volume. Interestingly, dolphins, who independently evolved for an aquatic lifestyle, have a similarly enlarged cerebellum taking up 15% of the total brain volume. This large cerebellum is hypothesized to allow for precise acoustic timing, acoustic processing, and echolocation abilities (e.g., for *Delphinus delphis*, *Tursiop truncatus,* see [23]). Future studies could explore the functions of the cerebellum in grey seals to further elucidate the role of this structure in vocal behavior and timing.

The weaned female grey seal brains showed the expected six-layer structure in the neocortex, which was of varying cortical thickness across the brain (min: 1 mm, max: 4.2 mm, *M* = 2.6 mm, *SD* = 0.7 mm; Supplement, *Figure S2-S10*). The mean cortical thickness was relatively close to that of harbor seals (2 mm range [18]), canines (2-3 mm range [24]), and humans (*M* = 2.5 mm [14]). The grey seal brains observed here (Supplement, *Figure S2-S10*) and the harbor seal brains examined in previous studies, showed high variation in both total cortical thickness across cortical areas (e.g., being very thick in gyri, very thin in sulci) and the thickness of specific layers across different cortical areas [18]. Given that grey seals have a neocortex characteristic of mammals, they could shed light on the role of different cortical areas and related laminar micro-circuits in mammalian vocal learning. This is particularly relevant since the most commonly studied vocal learners, songbirds, have a nuclear rather than laminar cortical organization making it more difficult to draw direct parallels to other vocal learning mammals such as humans.

Within the weaned female grey seal cortex, we found expression of FoxP2 - a gene well established as being important for human speech and songbird vocal learning [25,26] - in deeper layers (Supplement, *Figure S9-S10*). Future studies in grey seals could shed light on the role of FoxP2, and other key genes thought to be involved in vocal learning, in the cortex of mammalian vocal learners.

Conclusion

The current paper provides a first investigation into the neuroanatomy of the grey seal brain. By using information from dissected brain sections and MR images, we were able to create a neuroanatomical reference atlas, a standard brain template, 3D models of the weaned grey seal brain, and get a first glimpse of the neurogenetic properties of the grey seal brain. Based on the neuroanatomical information and brain templates provided here, future comparative studies of vocal learning in grey seals could employ techniques such as genetic mapping or diffusion tensor imaging to test hypotheses regarding the necessary and sufficient neural circuits involved in mammalian vocal learning. We believe that the study of vocal-learning pinnipeds will be quintessential to a complete understanding of the neurobiology of vocal learning since, as mammals, they can help bridging the gap from songbirds to research in humans and non-human primates [2].

Authors' contributions

NH and AR conceived and designed the study; NH drafted the manuscript; NH and LV prepared the neuroanatomical atlas and brain template; LV and AR coordinated the study and helped draft the manuscript; SCV conceived and analysed the histological data and edited the manuscript; NH, JM, CVR carried out the histology experiments and created the corresponding figures; SV, ASC and ARG facilitated access to the seals and extraction of the brains and provided advice; BCB facilitated access to the scanning facilities and provided advice. All authors edited the manuscript and gave final approval for publication and agreed to be held accountable for the work performed therein.

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References

1. Stansbury AL, Janik VM. 2019 Formant Modification through Vocal Production Learning in Gray Seals. *Curr. Biol.* **29**, 2244-2249.e4. (doi:10.1016/j.cub.2019.05.071)
2. Ravignani A, Fitch WT, Hanke FD, Heinrich T, Hurgitsch B, Kotz SA, Scharff C, Stoeger AS, Boer B de. 2016 What pinnipeds have to say about human speech, music, and the evolution of rhythm. *Front. Neurosci.* **10**. (doi:10.3389/fnins.2016.00274)
3. Janik VM, Slater PJB. 1997 *Vocal Learning in Mammals*. Elsevier Masson SAS. (doi:10.1016/S0065-3454(08)60377-0)
4. Lattenkamp EZ, Vernes SC. 2018 Vocal learning: a language-relevant trait in need of a broad cross-species approach. *Curr. Opin. Behav. Sci.* **21**, 209–215. (doi:10.1016/j.cobeha.2018.04.007)
5. Mars RB, Eichert N, Jbabdi S, Verhagen L, Rushworth MFS. 2018 Connectivity and the search for specializations in the language-capable brain. *Curr. Opin. Behav. Sci.* **21**, 19–26. (doi:10.1016/j.cobeha.2017.11.001)
6. Bininda-Emonds. 2000 Pinniped brain sizes. *Mar. Mammal Sci.* , 469–481.
7. Berta A. 2005 Integumentary and sensory systems. In *Marine mammals evolutionary biology*, pp. 132–164. Academic Press.
8. Dong W. 2008 Virtual cranial endocast of the oldest giant panda (Ailuropoda microta) reveals great similarity to that of its extant relative. *Naturwissenschaften* **95**, 1079–1083. (doi:10.1007/s00114-008-0419-3)
9. Thames RA, Robertson IAND, Flegel T, Henke D, O'Brien DP, Coate JR, Olby NJ. 2010 Development of a morphometric magnetic resonance image parameter suitable for distinguishing between normal dogs and dogs with cerebellar atrophy. *Vet. Radiol. Ultrasound* **51**, 246–253. (doi:10.1111/j.1740-8261.2009.01655.x)
10. Montie EW, Pussini N, Schneider GE, Battey TWK, Dennison S, Barakos J, Gulland F. 2009 Neuroanatomy and volumes of brain structures of a live California sea lion (Zalophus californianus) from magnetic resonance images. *Anat. Rec. (Hoboken).* **292**, 1523–1547. (doi:10.1002/ar.20937)
11. Zhang K, Sejnowski TJ. 2000 A universal scaling law between gray matter and white matter of cerebral cortex. *Proc. Natl. Acad. Sci.* **97**, 5621–5626. (doi:10.1073/pnas.090504197)

12. Matsuzawa J, Matsui M, Konishi T. 2001 Age-related Volumetric Changes of Brain Gray and White Matter in Healthy Infants and Children. *Cereb. Cortex* **11**, 335–342.
13. In press. Comparative Mammalian Brain Collections. See http://neurosciencelibrary.org/ (accessed on 25 February 2019).
14. Fischl B, Dale AM. 2000 Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc. Natl. Acad. Sci.* **97**, 11050–11055. (doi:10.1073/pnas.200033797)
15. Alderson AM, Diamantopoulos E, Downman CB. 1960 Auditory cortex of the seal (Phoca vitulina). *J. Anat.* **94**, 506–11.
16. Fish PA. 1895 A note on the cerebral fissuration of the seal. *J. Comp. Neurol.*
17. Langworthy OR, Hesser FH, Kolb LC, Rathbone HB, Rathbone JL. 1938 A physiological study of the cerebral cortex of the hair seal (Phoca vitulina). *J. Comp. Neurol.* **69**, 351–369. (doi:10.1002/cne.900690302)
18. Rioch DM. 1937 A physiological and histological study of the frontal cortex of the seal (phoca vitulina). *Biol. Bull.* **73**, 591–602. (doi:10.2307/1537617)
19. Bryant KL, Preuss TM. 2018 A Comparative Perspective on the Human Temporal Lobe. In *Digital Endocasts*, pp. 239–258. Tokyo: Springer Japan. (doi:10.1007/978-4-431-56582-6_16)
20. Pidoux L, Le Blanc P, Levenes C, Leblois A. 2018 A subcortical circuit linking the cerebellum to the basal ganglia engaged in vocal learning. *Elife* **7**, 3–5. (doi:10.7554/elife.32167)
21. Stoodley CJ. 2012 The cerebellum and cognition: Evidence from functional imaging studies. In *Cerebellum*, pp. 352–365. Springer. (doi:10.1007/s12311-011-0260-7)
22. Swanson LW. 1995 Mapping the human brain: past, present, and future. *Trends Neurosci.* **18**, 471–474.
23. Marino L, Rilling JK, Lin SK, Ridgway SH. 2000 Relative Volume of the Cerebellum. *Brain. Behav. Evol.* **30322**, 204–211.
24. Labounek R, Mai K, Mueller B, Ellinwood NM, Dickson P, Nestrasil I. 2019 In-vivo cortical thickness estimation from high-resolution T 1 w MRI scans in healthy and mucopolysaccharidosis affected dogs. In *2019 41st Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*, pp. 2848–2851. IEEE. (doi:10.1109/EMBC.2019.8856826)
25. Fisher SE, Scharff C. 2009 FOXP2 as a molecular window into speech and language. *Trends Genet.* **25**, 166–177. (doi:10.1016/j.tig.2009.03.002)
26. Vernes SC, Fisher SE. 2009 Unravelling neurogenetic networks implicated in developmental language disorders. *Biochem. Soc. Trans.* **37**, 1263–1269. (doi:10.1042/BST0371263)
27. Stephan H, Frahm H, Baron G. 1981 New and Revised Data on Volumes of Brain Structures in Insectivores and Primates. *Folia Primatol.* **35**, 1–29. (doi:10.1159/000155963)